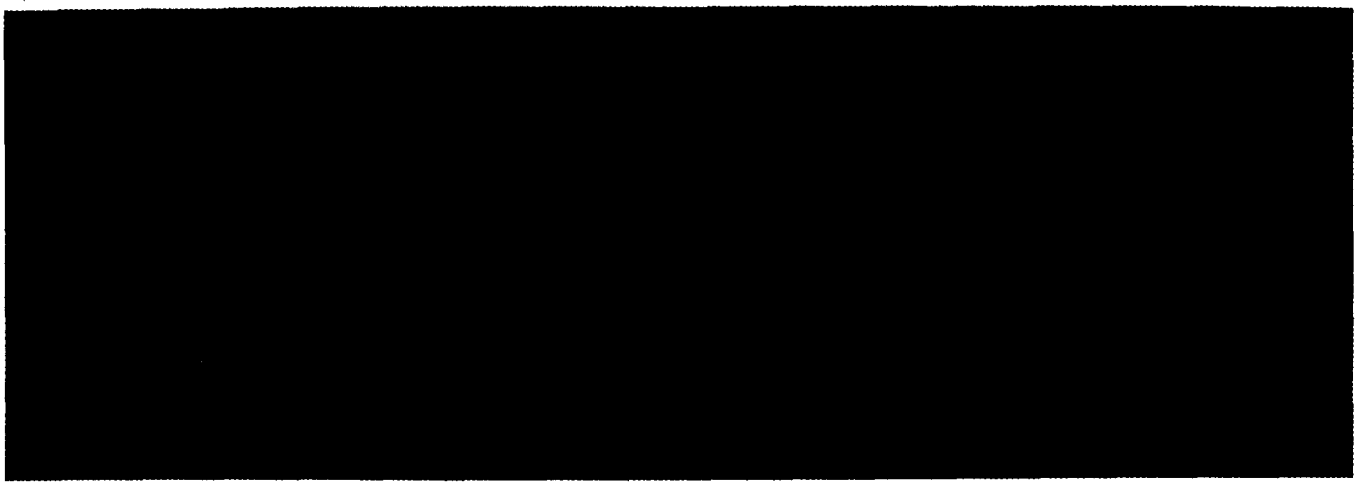

We may be on the threshold of a technology of untold importance in diagnostic and therapeutic medicine, says this Nobel geneticist, if we have the courage to move ahead despite the risks involved.

by JOSHUA LEDERBERG, Ph.D.

Although our theoretical understanding of the cell has been completely transformed in the last 30 years, there has not yet been a corresponding advance in the practical application of our knowledge to medicine. Indeed, very little in the practice of medicine (even of clinical genetics) is directly related to the fundamental knowledge that DNA has a bihelical structure.

Nonetheless, our faith remains steadfast that further theoretical understanding of viruses, the neoplastic cell, the aging cell, the immune response mechanism, and the aberrant chromosome, will bring far-reaching changes to medicine. The human benefit from such understanding will someday surely match the theoretical impact that DNA study has already made on cell biology.

These expectations for a possibly long-delayed future benefit have been heightened and accelerated by new findings that give us much greater technical ability to manipulate microbial DNA. New methods of DNA splicing have already opened up many lines of investigation into the structure of eukaryotic (higher life form) chromosomes.

We can now fragment animal or human DNA into perhaps a million segments and transfer a single segment to a bacterial host for study in a microcosm or for production of large quantities of a specific DNA segment. This allows more elaborate analysis than has ever been possible with the enormously complex, original, unfragmented source material.

This technique of gene implantation can also be used to transfer the genetic information for a given product from the cell of one species to that of another; and this is the direction, in my own view, that will lead to a technology of untold importance in diagnostic and therapeutic medicine: the ready production of an unlimited variety of human proteins. Analogous applications may be foreseen in fermentation processes for the cheap manufacture of essential nutrients and in the improvement of microbes for the production of antibiotics and special industrial chemicals.

In the face of such a revolution, the primary concern of researchers in the field has been the public hazards that such a technology may create. While we may indeed inherit a Promethean dilemma, public policy decision can lead to social good only if we are equally well-informed about the potential risks and benefits of further work on DNA splicing. If substantial risk can be identified, there is no doubt of the need for ethical and operational safety standards; the only question must be whether the form and implementation of such standards are adequate.

Too often, the "easy" way to handle such a problem is to invoke a formal regulatory statute, ignoring how well the actual bureaucratic enforcement or policing of the rules meets the intended balance of risks and benefits.

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The Author: Joshua Lederberg, Ph.D., shared the Nobel Prize in medicine and physiology in 1958 for his work in genetics. In 1946, he and Professor E. L. Tatum showed that bacterial cells can transfer genetic material from one cell to another. Subsequently, Dr. Lederberg and Dr. N. Zinder discovered the phenomenon of transduction, the carriage of genes by viruses. Dr. Lederberg has been professor of genetics at Stanford University since 1959.

Some thinkers feel that genetic engineering may open a Pandora's Box of troubles; yet with proper safeguards, further understanding of cellular processes can bring great benefits to mankind.

Before elaborating on the policy issues, it may be well for me to outline what is currently being done in DNA splicing, some promising applications, and also the risks of further work in this field.

DNA recombination, as the ultimate purpose of the sexual form of reproduction, is, of course, one of the major happenings in the natural world. Among higher life forms, DNA exchange is almost always limited to members of the same or closely related species. Bacteria and viruses, however, exhibit many exceptions to this rule, which perhaps reflects the fragility of the concept "species" when applied to these life forms.

For example, the entire group of enteric bacteria, including such forms as Shigella, Escherichia coli, Proteus, and Serratia, can exchange genetic fragments without special intervention. Our own experiments in genetic exchange would not seriously increase the risks already latent in that natural process.

Convenient Tools

An especially interesting and important level of genetic organization in bacteria is the plasmid: a bit of circular DNA that behaves like an extra chromosome and seems to survive in nature by viture of its easy transmissibility from one bacterial strain to another. Many different kinds of plasmids are known; in medicine, the most prominent are those which confer transmissible antibiotic resistance on human pathogens, notably staphylococci and some enteric pathogens such as Shigella.

These plasmids are a by-product of the evolution of their host organisms: the spread of antibiotic-resistance plasmids is the most formidable bacterial response yet to our widespread use of antibiotics. Other plasmids are undoubtedly involved in altering the pathogenicity and host-specificity of various bacteria; therefore, in simple self-defense, we must learn all we can about them, without delay.

Plasmids have also achieved special prominence for a technical reason—they are especially convenient tools for DNA splicing and for the transmission of DNA segments from one species to another, particularly in conjunction with another elegant tool: the R- (for restriction) enzyme. (The R-nucleases are widely distributed among cell types; they may be an important mechanism by which a cell fends off any "foreign" DNA while protecting its own.)

Stanley N. Cohen, M.D., of the Department of Medicine, Stanford University, has used an R-enzyme to simplify a naturally occurring plasmid to the point where it consisted of a small circle of DNA, embracing the minimum amount of genetic information needed to replicate, plus a single R-enzyme recognition site.

This artificial plasmid, pSC-101, has been an important tool in DNA splicing research. When exposed to R-enzyme, the circle is cut into a single open length with sticky ends. It is then possible to insert other sticky-ended pieces of DNA from divers sources into the plasmid, and finally to close it up with another enzyme, ligase. This process is the key to the convenient design and construction of new DNA molecules, which subsequently can be transferred to a bacterial host.

One important aspect of this research is that the new DNA does *not* have to come from the same bacterial species. For example, Dr. Cohen and his collaborators have already reported the successful transfer of DNA from a toad, Xenopus, to E. coli with evidence of the production of toad-like ribosomal nucleic acids in the modified bacteria.

In addition to these plasmids, bacterial viruses are being used in a similar fashion. Less elegantly, perhaps, segments of DNA from intact bacteria may also be used both for insertions and as the acceptors. So far, all of these techniques depend on the innate (and poorly understood) ability of bacterial cells to incorporate DNA furnished from without. There have been many published claims of similar phenomena with plant and animal cell acceptors, but to date the claims are unconfirmed.

The special power of the enzyme transfer techniques is that they depend on the basic chemical structure of DNA rather than on biological adaptation. Thus, laboratory manipulation may produce constructs that occur rarely, if ever, in the natural world. Most of these constructs would resemble hothouse plants, and be poorly adapted to competitive survival in the world outside the laboratory. But some, by chance, might be harbingers of new diseases, or the source of ecological upsets difficult to control—like the mongoose in Hawaii or the crabgrass in your lawn.

R-enzymes, mixed DNA, and acceptor bacteria surely bring about some DNA segment transfers in nature. Our knowledge of the extent of natural plasmid transmission among "unrelated" life forms was widened by recent discoveries of plasmids with extraordinarily broad host ranges. It is difficult, however, to assess just what *can* or *cannot* occur in nature.

Rapid Advancement

DNA splicing is, however, merely the most powerful of several artificial techniques which bring together more-or-less natural assemblages of DNA. Indeed, it may prove to be less powerful than older methods (sexual crossing, transduction with bacteriophage, DNA-mediated transformation) for special constructions involving larger complexes than the segments yielded by R-enzymes.

These methods, in turn, are an extension of the artificial breeding of domestic animals and plants. In any event, the most efficient application of DNA splicing requires intimate knowledge of the genetic structure of both the donor and the acceptor strains, for which breeding methods are important if not indispensable.

Perhaps the single most important conclusion is that this technology is just in its infancy but has already advanced far—and that it is simple enough to be applied in any laboratory which can handle pure bacterial cultures.

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Illustration for PRISM by PETER LLOYD

But it is just this simplicity, which makes for great convenience and speed of development, that has raised concern about the proliferation of such methods in the hands of people with perhaps less-than-mature professional and ethical judgment, and with insufficient skill to contain bacterial cultures in the laboratory.

Now that we have put the dangers of DNA splicing research into perspective, let us examine the promise that it holds. DNA segmentation and splicing is certain to play a vital role in the further domestication of microbes for such uses as the development of new antibiotics and the production of high-quality food protein supplements. However, the unique strength of this procedure is that it allows the large-scale production of gene products of a less easily domesticated species: man.

Human proteins already play a substantial role in medicine but a role which is hindered by scarce supply. Today, the most attractive candidates for such large-scale production are the human antibody globulins. Compared to the rare genetic defects in other proteins (as in the case of hemophilia), failure of error in the production of antibody globulin is quite prevalent and is known to play a major role in the breakdown of the body's defense against infectious disease, in autoimmune and allergic disease, and perhaps also in cancer.

The most comprehensive use for biosynthetic proteins would be in passive immunization against infectious disease. (Animal antisera were once used but had to be abandoned because of the anti-animal antibody that they provoked in man.) With wholesale production of biosynthetic proteins, passive globulin therapy could be targeted at those diseases for which either technical or social factors may bring about gaps in the protection provided by active immunization. Included in that group of diseases are influenza, hepatitis, smallpox, encephalitis, rubella, herpes, rabies and perhaps also trypanosomiasis, malaria, schistosomiasis, tuberculosis, leprosy, and many others.

Need for a Ready Defense

There is reason for special urgency in the development of a backup capability in passive immunization. Complacency about active immunization against diseases such as polio and the technical inadequacy of such vaccines as rubella and hepatitis have weakened our general posture of defense against viral pandemic. We have no assurance that the next influenza epidemic, slightly more virulent than the last one, will not take a million lives for lack of a ready defense.

A broader need for biosynthetic proteins lies in polyvalent prophylaxis for infants. The principal medical argument for breast feeding is that human milk provides the infant with colostrum and a continuing supply of maternal mixed globulins. In the future there might be a huge demand for polyvalent gamma globulin supplements for infants both in industrialized and in poorer countries. And an analogous veterinary use could bring about greater efficiency in livestock production.

Specific antibodies, of course, are already widely used as diagnostic reagents of high specificity and selectivity. But in sufficient quantity, blocking antibodies might also play a useful role in helping protect transplanted tissues and organs from immunological attack by the new host. Conversely, tissue-specific ligating antibodies, although not necessarily cytotoxic themselves, may be useful in enhancing the cell-specific toxicity of certain cancer drugs. Cell-specific reagents would also be invaluable in diagnosis and in the specific separation of human cell types for either diagnostic or therapeutic applications.

Besides the specific antibody globulins, a number of important, but less specific, proteins (complement, properdin) play a major part in defense against infection. Fibrinolysin (plasmin) and urokinase (plasminogen-activator) represent a group of enzymes that experimentally have shown promise in the control of embolism. Besides these human proteins, many human hormones are also discouragingly scarce for use in clinical trials. The list of such bioproducts could be extended substantially. And perhaps the most important products are those that remain to be discovered.

Of course, microbial biosynthesis may well be supplemented by organic synthesis in human and hybrid somatic cell cultures and by cell-free ribosomal synthesis with m-RNA extracted from natural sources or synthesized. Each of these methods has its own peculiar difficulties and hazards, and the whole field will be advanced most rapidly by using the best available methods for any given problem.

At present, perhaps a half-dozen bacterial species are

well enough understood to serve as prime vehicles in laboratory studies of DNA splicing. For safety and convenience, investigators have preferred not to use pathogenic forms. Yet many scientists are primarily concerned that DNA splicing may inadvertently generate a new pathogen inimicable to man or to some other species important to man's ecology. The most likely, but not necessarily the only, sources of such pathogenic genes are the organisms that most urgently need further study—the subtle and insidious killers not now amenable to medical treatment. These include slow virus infections that may be involved in a wide range of chronic diseases, including cancer and more familiar viruses, such as herpes, for which satisfactory vaccines are not available.

Speculating the Hazards

The public debate over DNA splicing has focused on the possible hazards of new microorganisms, and away from their utilitarian prospects. The most urgent concern has been the danger of introducing potentially cancer-causing DNA into common bacteria. While this hazard is clearly speculative, the general territory is so poorly understood that no one can argue against the need for cautious laboratory procedures. A number of workers—particularly those whose special experience or training has been in fields other than medical microbiology—have confessed giving almost no thought in the past to safety; some of them are now among the most zealous in demanding tighter regulation of such research. And that zeal has spread to create a sincere, almost frantic effort to ferret out and identify the most remote, conceivable hazards.

Viewed as a rather public soul-searching and self-education, these discussions are invaluable. The main danger is that some political imperative may forge these tentative questions into iron-clad regulations which will be with us long after their origins have been forgotten. After all, similar questions can be raised about the widest range of human activities: should it be lawful to keep domestic cats when we suspect that they harbor toxoplasmosis, and possibly leukemia as well? Similarly, what assurance do we have that artificial pollination will not produce a weed that could ruin the wheat crop a decade from now? Closer to home, should we forbid international travel simply because our quarantine procedures do not guarantee that exotic diseases will be kept out?

For each of these cases, and many more, the apparently innocuous doctrine, "As long as there is any risk, don't do it!" can only bring a loss to human welfare. We must instead make every feasible effort to assess both the risks and the benefits of a given course of action—only then will we be able to find the optimal balance. But individuals can hardly determine the best policy about their own future—including their expectations for what medicine will offer for the infirmities of their own later years—without expert assessment.

Such assessments are difficult, problematical, and controversial. But a committee of the National Academy of Sciences has made some headway in trying to classify different categories of hazard. Where such hazard is reasonably predictable, the committee has recommended laboratory containment precautions akin to those appropriate for known pathogens. This applies, for example, to experiments in the recombination of known tumor virus DNA with bacterial plasmids.

For more conjectural hazards, such as the introduction of antibiotic resistance into common, non-pathogenic species, the high security requirements recommended by the committee may be an inordinate burden for laboratories (who, in fact, will pay for them?) in relation to the prospective gains. The best strategy in such a case seems to be the development of safe vectors: plasmids and bacteria engineered so that they have little chance of surviving outside the laboratory. In fact, in the long run this is a safer procedure than relying upon the uncertainty of human compliance with fixed rules and regulations.

Remaining controversies in this area center upon rather complicated analyses of the most remote risks. Given some additional time, most research institutions will work out their own reasonable plans, based on the national guidelines. A premature imposition of external regulation will not only frustrate useful research, but will also hinder that research which is needed to more accurately assess the dangers. Those who consider themselves guardians of the public safety must count the costs to the public health of *impeding* research, as well as the speculative *hazards* of research.

Society's Consent

This partly voluntary approach will not assure absolutely that no foolish experiment is ever attempted. But the history of human institutions should suffice to show that *no* system of sanctions can achieve such a goal. The human species is inevitably attended by contaminating and parasitic microbes—the person suffering from an enteric infection who fails to wash his hands or the influenza victim who insists on going to work is behaving unethically and to the peril of his fellows. But we would scarcely invoke serious regulatory sanctions in preference to public education, except where there is an unusual public risk with some attendant evidence that an enforced quarantine would be effective.

Senator Edward Kennedy (D-Mass.) has remarked that society must give its informed consent to technological innovation. The power of the purse is enough to enforce that doctrine; nor can there be any quarrel with it on ethical grounds. Informed consent surely includes knowing the hazards of saying no to the prospects of significant medical advances. DNA splicing research, far from being an idle scientific toy or the basis for expensive and specialized aid to the privileged few, promises some of the most pervasive benefits for the public health since the discovery and promulgation of antibiotics.